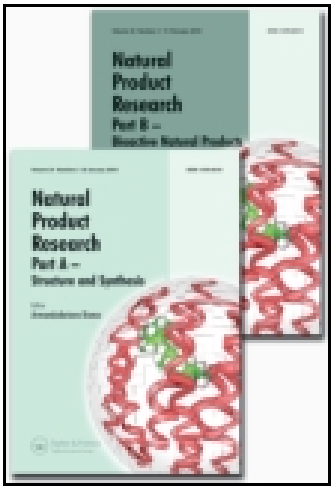


Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Separation and elucidation of anthocyanins in the fruit of mockstrawberry (Duchesnea indica Focke)

Chuanguang Qin [a], Yang Li [a], Ruijie Zhang [a], Weining Niu [a] & Yan Ding [a]
[a] Faculty of Life Science, Northwestern Polytechnical University, Xi'an 710072, Shaanxi, China
Published online: 22 Sep 2010.

To cite this article: Chuanguang Qin, Yang Li, Ruijie Zhang, Weining Niu & Yan Ding (2009) Separation and elucidation of anthocyanins in the fruit of mockstrawberry (Duchesnea indica Focke), Natural Product Research: Formerly Natural Product Letters, 23:17, 1589-1598, DOI: 10.1080/14786410802496903

To link to this article: http://dx.doi.org/10.1080/14786410802496903

Natural Product Research
Vol. 23, No. 17, 20 November 2009, 1589–1598

Separation and elucidation of anthocyanins in the fruit of mockstrawberry (*Duchesnea indica* Focke)

Chuanguang Qin*, Yang Li, Ruijie Zhang, Weining Niu and Yan Ding

Faculty of Life Science, Northwestern Polytechnical University, Xi'an 710072, Shaanxi, China

(*Received 18 June 2008; final version received 18 September 2008*)

Anthocyanin pigments in the fruit of mockstrawberry (*Duchesnea indica* Focke), were extracted with 0.1% HCl in ethanol, and the crude anthocyanin extract was purified by a C18 Sep-Pak cartridge open-column chromatography. High-performance liquid chromatography (HPLC) with photodiode array detection and mass spectrometry (MS) was applied for the isolation and composition analysis of anthocyanins in the fruit of mockstrawberry and their aglycones from acid hydrolysis. Three anthocyanins were found in the fruit of mock-strawberry and they were identified as cyanidin 3-*O*-rutinoside (61%), peonidin 3-*O*-rutinoside (34%), and petunidin 3-*O*-rutinoside (5%), respectively, by spectroscopic methods (UV-vis and MS). The two major anthocyanins were isolated by preparative HPLC, and their chemical structures were further characterised by H^1 NMR. On the basis of chromatographic data, the total anthocyanin content was 205 mg g^{-1} of the fresh fruit of mockstrawberry.

Keywords: anthocyanins; mockstrawberry; *Duchesnea indica* Focke; natural colourants; composition analysis

1. Introduction

Anthocyanins are a group of over 500 compounds that are largely responsible for the many blue, purple, red, and orange colours exhibited by plants (Andersen & Jordheim, 2006). Chemically, they are classified as flavonoids and have a molecular structure based on the 2-phenylbenzopyrylium (or flavylium) cation. Although anthocyanins have been widely studied because of their central role in determining the colour of plant organs, they also have biochemical properties that are believed to be beneficial to human health. Like other flavonoids, anthocyanins are polyphenolic compounds and exhibit substantial *in vitro* antioxidant capacity (Kahkonen & Heinonen, 2003; Konga, Chiaa, Goha, Chiaa, & Brouillardb 2003). Additionally, anthocyanins have been shown to have a range of potential therapeutic properties such as anti-inflammatory, cancer chemoprevention, antiobesity, and vasoprotection (Chen et al., 2006; Cooke, Steward, Gescher, & Marczylo, 2005; Hou, 2003; Jayaprakasam, Vareed, Olson, & Nair, 2005; Katsube, Iwashita, Tsushida, Yamaki, & Kobori, 2003; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003; Xu, Ikeda, & Yamori, 2005). Since anthocyanins accumulate to high concentrations in some foods, for example, berry fruit, consumption ranging up to several 100 mg per serving can

*Corresponding author. Email: qinchg@nwpu.edu.cn

ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786410802496903
http://www.informaworld.com

be achieved. However, despite the identified *in vitro* therapeutic properties of anthocyanins and their widespread presence in the diet, studies to date indicate that the apparent bioavailability is very low compared with other polyphenolics and flavonoids (Manach, Williamson, Morand, Scalbert, & Remesy, 2005; McGhie, Ainge, Barnett, Cooney, & Jensen, 2003; Sÿkerget, et al., 2005), which suggest that anthocyanins might not exhibit a therapeutic effect when consumed as part of the diet. As a class of compounds, anthocyanins have a diverse range of molecular structures that are likely to play important roles in determining their biological activity and associated factors such as bioavailability and metabolism. Berry fruits, such as raspberry, blackberry, blueberry, mulberry, strawberry, bayberry, and black raspberry, in addition to hybrid berries like marionberry, boysenberry, and tayberry, were recently reported to contribute a range of anthocyanins, and each type of berry fruit has a particular spectrum of anthocyanins (Chaovanalikit, Thompson, & Wrolstad, 2004; Longo & Vasapollo, 2005a, 2006; McGhie, Rowan, & Edwards, 2006; Mullen, Lean, & Crozier, 2002; Tian, Giusti, Stoner, & Schwartz, 2001, 2006). We are particularly interested in the anthocyanin composition in the fruit of the mockstrawberry (*Duchesnea indica* Focke), which is a kind of traditional Chinese herbal medicine and has been used in folk remedies for the treatment of pains, carbuncles, inflammations, and cancer for a long time. In this article, the profile of anthocyanins in the fruit of mockstrawberry was reported for their potential application as natural colourants and antioxidant agents by food, pharmaceutical, and cosmetic industries.

2. Materials and methods

2.1. *Chemicals and standards*

HPLC grade solvents, such as methanol, acetonitrile, isopropanol and chloroform etc., were purchased from Fisher Scientific (NJ, USA). Trifluoroacetic acid (TFA) and tetrahydrofuran of HPLC grade were purchased from Tedia Co. Inc. (Fairfield, OH, USA). d-Trifluoroacetic acid (CF_3COOD, 99.8%), d^4-methanol (CD_3OD, 99.8%) and trimethylsilicane (TMS) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and cyanidin 3-*O*-rutinoside was from Extrasynthese (Genay, France). Deionised water was used to prepare all solutions.

2.2. *Sample collection*

Wild growing mockstrawberry fruits (Figure 1(a)) were hand-picked in Xi'an, China, during May 2007, placed in polyethylene bags, and stored at −20°C until use. The plant was classified at the Department of Biology, Northwest University, China, as *Duchesnea indica* Focke.

2.3. *Extraction of anthocyanins*

The milled fresh mockstrawberry fruits (500 g) were extracted in the dark by stirring with 1000 mL of 0.1% HCl (v/v) in ethanol for 20 h at room temperature. The samples were filtered on a Buchner funnel, and the solid residue was washed with an additional 50 mL of 0.1% HCl (v/v) in ethanol. Filtrates were combined and dried using a rotary evaporator at 30°C. The remaining solid was dissolved in 0.01% HCl (v/v) in deionised water and successively purified.

Figure 1. Pigment from the fruit of mockstrawberry (*Duchesnea indica* (Andr.) Focke).

2.4. *Purification of anthocyanins*

The anthocyanin aqueous solution obtained from the extraction procedure described earlier was passed through a 5 g sorbent weight C-18 Sep-Pak cartridge (Waters Corp., Milford, MA), previously activated with methanol followed by 0.01% aqueous HCl (v/v). Anthocyanins and other polyphenolics were adsorbed onto the Sep-Pak column, while sugars, acids, and other water soluble compounds were removed by washing the cartridge with 2 volumes of 0.01% aqueous HCl (v/v). Less polar polyphenolics were subsequently eluted with 2 volumes of ethyl acetate. Anthocyanins were then eluted with methanol containing 0.01% HCl (v/v). The acidified methanol solution was evaporated using a rotary evaporator at 30°C. The remaining solid was dissolved in 0.01% HCl (v/v) aqueous solution to have a known concentration solution (2 mg mL^{-1}) and immediately analysed. This solution (Figure 1(b)) was stored at −20°C until used for successive acid and alkaline hydrolyses.

2.5. *Acid hydrolysis of anthocyanins*

First, 5 mL of 2 N HCl was added to 1 mL of the purified anthocyanin solution (2 mg mL^{-1}) in a screw-cap test tube, flushed with nitrogen, and capped. The pigments were hydrolysed for 2 h at 100°C; then, the solution was immediately cooled in an ice bath (Ordaz-Galindo, Wesche-Ebeling, Wrolstad, Rodriguez-Saona, & Argaiz-Jamet, 1999). The hydrolysate was purified by using a 0.5 g sorbent weight C-18 Sep-Pak cartridge (Waters) as previously described.

2.6. *HPLC–PAD–MS analysis*

The high-performance liquid chromatography (HPLC)–photodiode array detection (PAD)–mass spectrometry (MS) analyses were performed using a Waters 2696 separation

module equipped with a 996 photodiode array detector (PAD) coupled to a mass spectrometer (quadrupole analyser) equipped with an electrospray ionisation interface. Chromatographic separation was carried out using a 250 × 4.6 mm i.d., 5 μm Kromasil C18 column (No.: NC-2546-06251151) with a 4 × 3 mm i.d. Phenomenex C18 guard cartridge, both thermostated at 32°C. The mobile phase was composed of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) at a flow rate of 1 mL min^{-1}. The following gradient was utilised: 0 min, 10% B; 0–2 min, 10% B; 2–35 min, 10–90% B; 35–40 min, 90–100% B; 40–60 min, 100% B. Absorbance spectra were recorded every 2 s, between 200 and 600 nm, with a bandwidth of 4 nm, and chromatograms were acquired at 520, 440, 310, and 280 nm. MS parameters were as follows: capillary voltage, 4000 V; fragmentor, 160 V; drying gas temperature, 350°C; gas flow (N_2), 10 L min^{-1}; nebuliser pressure, 50 psig. The instrument was operated in positive ion mode scanning from m/z 100 to 800 at a scan rate of 1.43 s per cycle. The wavelength used for quantification was 520 nm (De Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2002; Hong & Wrolstad, 1990). The calibration curve was produced by the integration of absorption peaks generated from the analysis of dilution series of cyanidin 3-*O*-rutinoside.

2.7. *Preparative HPLC*

The major anthocyanins were isolated by a preparative HPLC system consisting of a Shimadzu model LC-8A pump, a Shimadzu SCL-10A VP system controller, a manual injector fitted with a 1 mL sample loop, and a Shimadzu model SPD-10A UV/vis detector equipped with a preparative flow cell. A 250 × 21.2 mm i.d., 7 μm Zorbax Stable Bond-C18 preparative column, coupled to a 50 × 21.2 mm i.d., 5 μm Zorbax Stable Bond-C18 guard column, was used for the separation and isolation of anthocyanins. The mobile phase was formic acid/water/methanol (10 : 75 : 15 v/v). The isocratic flow rate was 20 mL min^{-1}, and the detector was set at 520 nm. The anthocyanin fractions of interest were isolated, and their purity was checked by analytical HPLC–PAD–MS analysis, as previously described. Each fraction was evaporated using a rotary evaporator at 30°C to completely remove the methanol and then loaded on a 5 g sorbent weight C-18 Sep-Pak cartridge (Waters) previously activated with methanol followed by 0.01% aqueous HCl (v/v). The cartridge was rinsed with 5 volumes of 0.01% aqueous HCl (v/v), and the adsorbed anthocyanin was eluted with 0.1% HCl (v/v) in methanol. The eluent solution was evaporated to dryness using a rotary evaporator at 30°C, and the resulting solid was resolubilised with the suitable solvent for NMR analysis.

2.8. *NMR analysis*

^{1}H NMR spectral data were recorded on an INOVA-400 instrument (Varian Inc., Palo Alto, CA, USA) operating at 400 MHz in solvent CD_3OD–CF_3COOD (9 : 1) with TMS as internal standard. Sample temperature was stabilised at 25°C (Longo & Vasapollo, 2005b; Longo, Vasapollo, & Rescio, 2005).

3. Results and discussion

The anthocyanin composition of the fruit of mockstrawberry was determined by means of HPLC–PAD–MS analysis. The HPLC profile recorded at 520 nm (Figure 2(a)) indicated

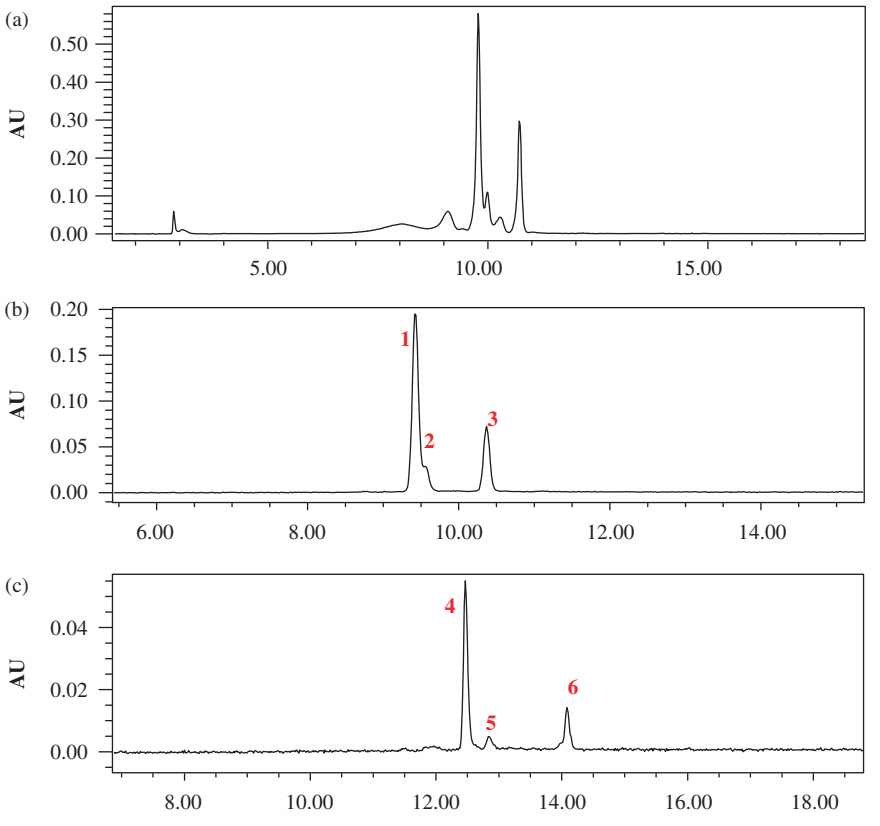
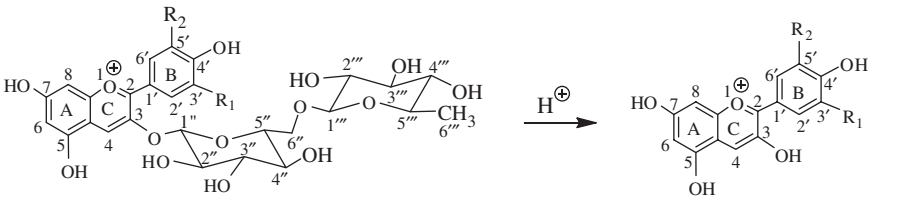


Figure 2. HPLC–PAD chromatogram recorded at 520 nm corresponding to (a) the crude extract from the fruit of mockstrawberry; (b) the purified anthocyanins from the fruit of mockstrawberry; (c) the purified anthocyanidins from the mockstrawberry anthocyanins after acid hydrolysis.

that there were six components in crude extract from the fruit of mockstrawberry at retention time range of 8–11 min, which absorb in this region of the visible region. After purification through the C-18 column of Sep-Pak cartridge (Waters Corp., Milford, MA), the chromatogram of the purified anthocyanin extract from the fruit of mockstrawberry, recorded at 520 nm, was shown in Figure 2(b). As can be seen, there are only three peaks in the chromatogram, indicating the presence of three different anthocyanins in the fruit of mockstrawberry. The chromatogram of the purified product after acid hydrolysis of the anthocyanin extract, recorded at 520 nm (Figure 2(c)), showed that three different aglycones could be obtained from the three anthocyanins in the mockstrawberry fruit. These three anthocyanins and their corresponding aglycones, the structures of which were shown in Figure 3, were identified by comparison of HPLC retention times, elution order, photodiode array UV/vis spectroscopic, and ESI–MS spectrometric data (Table 1).

The two major anthocyanins corresponding to peaks **1** and **3** (Figure 2(b)) represented about 61 and 34%, respectively, of the total peak area revealed at 520 nm. Peak **1** was identified as cyanidin 3-*O*-rutinoside on the basis of its λ_{max} of 516 nm and a mass spectrum comprising a M^+ at *m*/*z* 595 and two fragment ions at *m*/*z* 449 and 287 resulting from the loss of the deoxyglucose ($M^+ - 142$) and the rutinose ($M^+ - 308$), respectively.



1 R_1=OH, R_2=H
2 R_1=OH, R_2=OCH3
3 R_1=OCH_3, R_2=H

4 R_1= OH, R_2= H
5 R_1= OH, R_2= OCH_3
6 R_1= OCH_3, R_2= H

Figure 3. Chemical structures of the anthocyanins identified in the fruit of mockstrawberry and their aglycones after acid hydrolysis: **1**, cyanidin 3-*O*-rutinoside (Cy-3-Rut); **2**, petunidin 3-*O*-rutinoside (Pt-3-Rut); **3**, peonidin 3-*O*-rutinoside (Pn-3-Rut); **4**, cyanidin (Cy); **5**, petunidin (Pt); **6**, peonidin (Pn).

Table 1. Chromatographic, spectroscopic and spectrometric characteristics of the anthocyanins found in the fruit of mockstrawberry and their aglycones after acid hydrolysis.

Peak no. (Figure 2)	t_R (min)	λ_{max} (nm)	$A_{(440nm)}/A_{(\lambda_{max})}$	M (calcd.)	M^+ (found)	M^+-Rut (m/z)	Peak assignment
1	9.6	518 (281)	30.3	595.17	595.22	287.20	Cy-3-Rut
2	9.8	526 (281)	33.1	625.18	625.24	317.35	Pt-3-Rut
3	10.6	516 (280)	32.2	609.18	609.25	301.3	Pn-3-Rut
4	12.5	525 (270)	25.8	287.1	287.2	–	Cyanidin (Cy)
5	12.8	525 (255)	26.7	317.1	317.3	–	Petunidin (Pt)
6	14.1	524 (268)	30.5	301.1	301.2	–	Peonidin (Pn)

The aglycone corresponded to the molecular ion of cyanidin (**4**). The ESI–MS profiles of the peak **3** presented the molecular ions M^+ at m/z 609 and the fragment ions at m/z 467 and 301 resulting from the loss of the deoxyglucose ($M^+ - 142$) and rutinose ($M^+ - 308$), respectively. The aglycone corresponded to the molecular ion of peonidin (**6**). Peak **3** was therefore identified as peonidin 3-*O*-rutinoside. The minor anthocyanin (peak **2**, around 5%) had a λ_{max} of 526 nm and a mass spectrum consisting of a M^+ at m/z 625 and fragment ions at m/z 483 and 317 resulting from the loss of the deoxyglucose ($M^+ - 142$) and rutinose ($M^+ - 308$), respectively. The aglycone corresponded to the molecular ion of petunidin (**5**). Anthocyanin (**2**) was therefore identified as petunidin 3-*O*-rutinoside. The UV/vis absorbance spectra of these compounds (Figure 4) confirmed their identity.

The Abs440/Absλ_{max} ratio values calculated for each anthocyanin, ranging from 31 to 32%, indicated a substitution in the C-3 position of the flavylium ring (Giusti, Rodriguez-Saona, & Wrolstad, 1999). It is well known that anthocyanins with glycosidic substitutions at position 3 exhibit a ratio of the absorbance at 400–440 nm to the absorbance at the visible maximum wavelength (520 nm) that is almost twice as large as for anthocyanins with glycosidic substitution at position 5 or both 3 and 5 (Harborne, 1976). In addition, the obtained Abs280/Absλ_{max} (67–100%) and Abs310/Absλ_{max} (13–22%) ratios confirmed that mockstrawberry anthocyanins were simple anthocyanin molecules without acylation of glycoside with aromatic acids (Mozetic, Trebse, & Hribar, 2002; Woodall & Stewart, 1998).

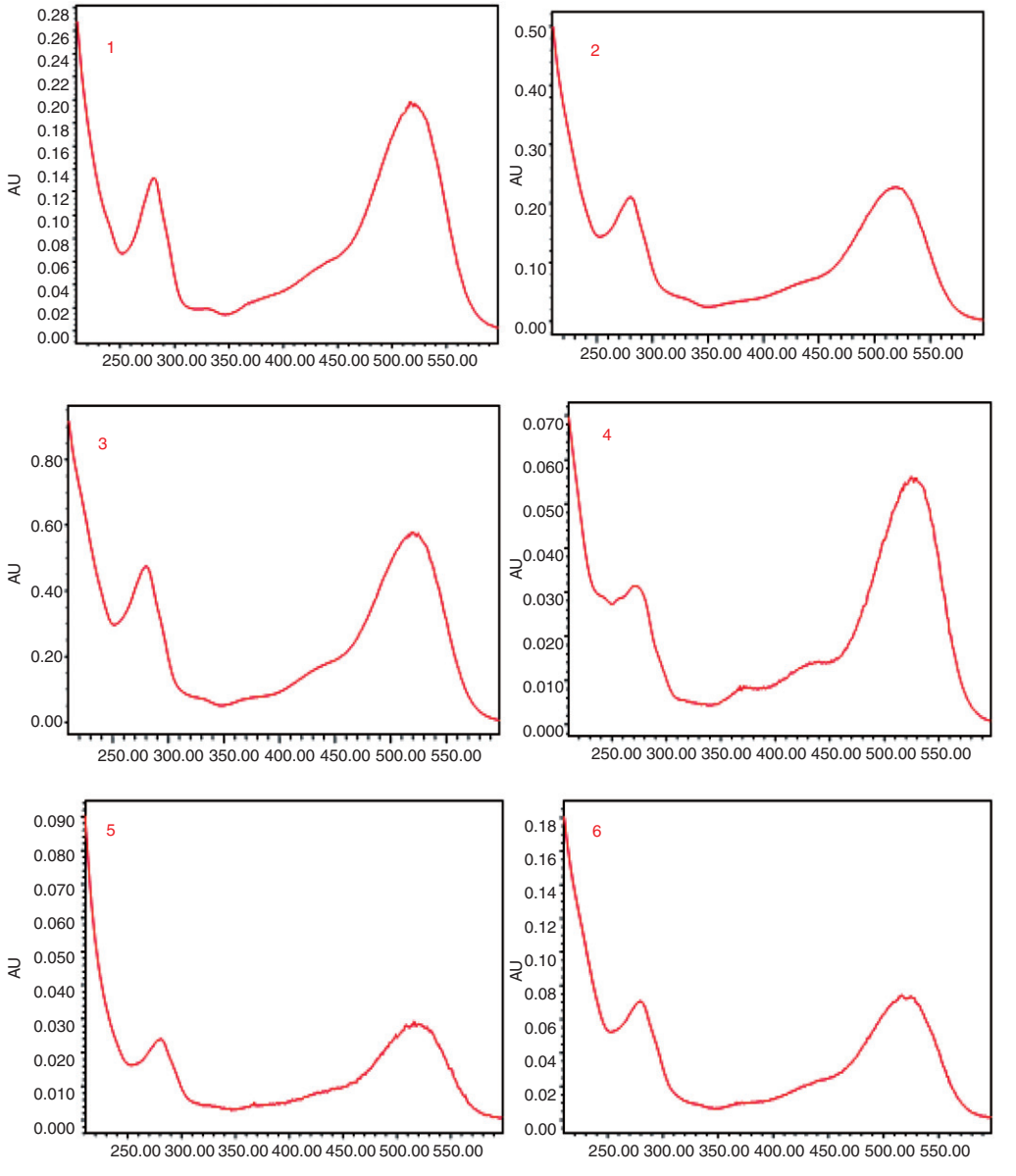


Figure 4. UV–visible spectrum of peaks **1**–**6** recorded with a PAD from 200 to 600 nm: 1. peak **1**; 2. peak **2**; 3. peak **3**; 4. peak **4**; 5. peak **5**; 6. peak **6**.

Acid hydrolysis of the purified anthocyanins produced three peaks (**4**, **5** and **6**), as shown in the chromatogram of Figure 2(c). The ESI–MS profiles of these compounds presented their molecular ions M^+ at *m*/*z* 287 (**4**), 317 (**5**) and 301 (**6**), corresponding to the molecular ions of cyanidin, petunidin, and peonidin, respectively (Pridham, 1964). The absorbance spectra of these compounds confirmed their identity (see Figure 4).

Table 2. ^{1}H NMR spectroscopic data of two anthocyanins (**1** and **3**) isolated from the extract of mockstrawberry fruit (δ in CD_3OD–CF_3COOD (9 : 1) at 25°C).

Anthocyanin	**1** δ (J in Hz)	**3** δ (J in Hz)
Aglycone		
H-4	9.01 s	8.95 s
H-6	6.65 d (2.1)	6.69 d (1.8)
H-8	6.89 brs	6.90 brs
H-2′	8.06 d (2.4)	8.05 d (2.5)
H-5′	7.05 d (8.5)	7.03 d (8.7)
H-6′	8.29 dd (2.5, 8.8)	8.30 dd (2.4, 8.5)
O–CH_3		3.92 s
3-Glucosyl		
H-1″	5.31 d (7.8)	5.30 d (7.8)
H-2″, H-3″, H-4″, H-5″, H-6″	3.20–3.55 *m*	3.18–3.52 *m*

Among the three pigments detected in the fruit of mockstrawberry, the two major anthocyanins (compounds **1** and **3**, Figure 2(b)) were isolated by preparative HPLC and their identity and structure were confirmed on the basis of ^{1}H NMR spectroscopic data. The chemical shifts (δ) obtained from the ^{1}H NMR analysis of anthocyanins **1** and **3** were reported in Table 2. Signals in the downfield of the spectra between δ 6.7 and 9.0 ppm were clearly attributable to the aromatic protons (A and B rings) of the aglycone molecule as previously reported (Frøytlog, Slimestad, & Andersen, 1998; Kuskoski et al., 2003) for these compounds. The signal doublets at δ 5.3 corresponded to the protons on the anomeric carbon from the glucose residues, confirming that they were in position C-3 as also indicated by the Abs440/Absλ_{max} ratio values. The β-configuration of this moiety was confirmed from the magnitude ($J = 7.6$ Hz) of the $J_{1''2''}$ coupling constant in the ^{1}H NMR spectra (Gonnett & Fenet, 2000; Pale, Nacro, Vanhaelen, & Vanhaelen-Fastrè, 1997). The spectrum of the compound presented a doublet at δ 5.29 (d, $J = 7.6$ Hz, H-1 glucose) confirming the presence of glucose as sugar moiety (Fossen, Øvstedal, Slimestad, & Andersen, 2003; Frøytlog et al., 1998). It was not possible to confirm by means of NMR analysis the identity of the anthocyanins (**2**) (Figure 2(b)), which were characterised as rutinoside derivative of petunidin by means of HPLC–PAD–MS analysis; their low concentration in the mockstrawberry extract did not enable us to preparatively isolate this minor anthocyanin for their NMR characterisation.

4. Conclusion

The total amount of anthocyanins in the fruit of mockstrawberry, determined on the cyanidin 3-*O*-rutinoside basis, was 205 mg g^{-1} of stoned fresh fruit of mockstrawberry. Cyanidin 3-*O*-rutinoside was the most predominant anthocyanin (125 mg g^{-1}), followed by peonidin 3-*O*-rutinoside (70 mg g^{-1}). The amount of petunidin 3-*O*-rutinoside was 10 mg g^{-1}. To our knowledge, this is the first time that the anthocyanin composition of mockstrawberry fruit has been described.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (20672086), China Postdoctoral Science Foundation (20070411144) and the Scientific Research Foundation for Returned Overseas Chinese Scholars, State Personnel Ministry of China (2007).

References

Andersen, O.M., & Jordheim, M. (2006). The anthocyanins. In O.M. Andersen & K.R. Markham (Eds.), *Flavonoids chemistry, biochemistry and applications* (pp. 471–551). Boca Raton, FL: CRC Press, Taylor and Francis.

Chaovanalikit, A., Thompson, M.M., & Wrolstad, R.E. (2004). Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). *Journal of Agricultural and Food Chemistry, 52*, 848–852.

Chen, P.N., Chu, S.C., Chiou, H.L., Kuo, W.H., Chiang, C.L., & Hsieh, Y.S. (2006). Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-rutinoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. *Cancer Letters, 235*, 248–259.

Cooke, D., Steward, P.W., Gescher, J.A., & Marczylo, T. (2005). Anthocyans from fruits and vegetables – Does bright colour signal cancer chemopreventive activity? *European Journal of Cancer, 41*, 1931–1940.

De Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J.C. (2002). LC-MS analysis of anthocyanins from purple corn cob. *Journal of the Science of Food and Agriculture, 82*, 1003–1006.

Fossen, T., Øvstedal, D.O., Slimestad, R., & Andersen, Ø.M. (2003). Anthocyanins from a Norwegian potato cultivar. *Food Chemistry, 81*, 433–437.

Frøytlog, C., Slimestad, R., & Andersen, Ø.M. (1998). Combination of chromatographic techniques for the preparative isolation of anthocyanins – Applied on blackcurrant (*Ribes nigrum*) fruits. *Journal of Chromatography A, 825*, 89–95.

Gonnett, J.F., & Fenet, B. (2000). Cyclamen red colors based on a macrocyclic anthocyanin in Carnation flowers. *Journal of Agricultural and Food Chemistry, 48*, 22–26.

Giusti, M.M., Rodriguez-Saona, L.E., & Wrolstad, R.E. (1999). Molar absorptivity and color characteristics of acylated and nonacylated peonidin-based anthocyanins. *Journal of Agricultural and Food Chemistry, 47*, 4631–4637.

Harborne, J.B. (1976). *Comparative biochemistry of the flavonoids*. New York: Academic Press.

Hong, V., & Wrolstad, R.E. (1990). Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *Journal of Agricultural and Food Chemistry, 38*, 708–715.

Hou, D.X. (2003). Potential mechanisms of cancer chemoprevention by anthocyanins. *Current Molecular Medicine, 3*, 149–159.

Jayaprakasam, B., Vareed, S.K., Olson, L.K., & Nair, M.G. (2005). Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. *Journal of Agricultural and Food Chemistry, 53*, 28–31.

Kahkonen, M.P., & Heinonen, M. (2003). Antioxidant activity of anthocyanins and their aglycons. *Journal of Agricultural and Food Chemistry, 51*, 628–633.

Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K., & Kobori, M. (2003). Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *Journal of Agricultural and Food Chemistry, 51*, 68–75.

Konga, J.M., Chiaa, L.S., Goha, N.K., Chiaa, T.F., & Brouillardb, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry, 64*, 923–933.

Kuskoski, E.M., Vega, J.M., Rios, J.J., Fett, R., Troncoso, A.M., & Asuero, A.G. (2003). Characterization of anthocyanins from the fruits of baguacüu (*Eugenia umbelliflora* Berg). *Journal of Agricultural and Food Chemistry, 51*, 5450–5454.

Longo, L., & Vasapollo, G. (2005a). Determination of anthocyanins in *Ruscus aculeatus* L. berries. *Journal of Agricultural and Food Chemistry, 53*, 475–479.

Longo, L., & Vasapollo, G. (2005b). Anthocyanins from bay (*Laurus nobilis* L.) berries. *Journal of Agricultural and Food Chemistry, 53*, 8063–8067.

Longo, L., & Vasapollo, G. (2006). Extraction and identification of anthocyanins from *Smilax aspera* L. berries. *Food Chemistry, 94*, 226–231.

Longo, L., Vasapollo, G., & Rescio, L. (2005). Identification of anthocyanins in *Rhamnus alaternus* L. berries. *Journal of Agricultural and Food Chemistry, 53*, 1723–1727.

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. 1. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition, 81*, 230S–242S.

McGhie, T.K., Ainge, G.D., Barnett, L.E., Cooney, J.M., & Jensen, D.J. (2003). Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolised by both humans and rats. *Journal of Agricultural and Food Chemistry, 51*, 4539–4548.

McGhie, T.K., Rowan, D.R., & Edwards, P.J. (2006). Structural identification of two major anthocyanin components of boysenberry by NMR Spectroscopy. *Journal of Agricultural and Food Chemistry, 54*, 8756–8761.

Mozetic, B., Trebse, P., & Hribar, J. (2002). Determination and quantification of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (*Prunusa* V*ium* L.) from Nova Gorica Region (Slovenia). *Food Technology and Biotechnology, 40*, 207–212.

Mullen, W., Lean, M.E.J., & Crozier, A. (2002). Rapid characterization of anthocyanins in red raspberry fruit by high-performance liquid chromatography coupled to single quadrupole mass spectrometry. *Journal of Chromatography A, 966*, 63–70.

Ordaz-Galindo, A., Wesche-Ebeling, P., Wrolstad, R.E., Rodriguez-Saona, L., & Argaiz-Jamet, A. (1999). Purification and identification of Capulin (*Prunus serotina* Ehrh) anthocyanins. *Food Chemistry, 65*, 201–206.

Pale, E., Nacro, M., Vanhaelen, M., & Vanhaelen-Fastrè, R. (1997). Anthocyanins from bambara groundnut (*Vigna subterranea*). *Journal of Agricultural and Food Chemistry, 45*, 3359–3361.

Pridham, J.B. (1964). *Paper electrophoresis of phenolic compounds. Methods of polyphenol chemistry*. New York: Pergamon Press.

Sÿkerget, M., Kotnik, P., Hadolin, M., Hraš, A.R., Simonič, M., & Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry, 89*, 191–198.

Tian, Q.G., Giusti, M.M., Stoner, D.G., & Schwartz, J.S. (2001). Identification of anthocyanins in berries by narrow-bore high-performance liquid chromatography with electrospray ionization detection. *Journal of Agricultural and Food Chemistry, 49*, 3987–3992.

Tian, Q.G., Giusti, M.M., Stoner, D.G., & Schwartz, J.S. (2006). Characterization of a new anthocyanin in black raspberries (*Rubus occidentalis*) by liquid chromatography electrospray ionization tandem mass spectrometry. *Food Chemistry, 94*, 465–468.

Tsuda, T., Horio, F., Uchida, K., Aoki, H., & Osawa, T. (2003). Dietary cyanidin 3-*O*-beta-D-rutinoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *Journal of Nutrition, 133*, 2125–2130.

Woodall, G.S., & Stewart, G.R. (1998). Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*? *Journal of Experimental Botany, 49*, 1447–1450.

Xu, J.W., Ikeda, K., & Yamori, Y. (2005). Cyandin-3-rutinoside regulates phosphorylation of endothelial nitric oxide synthase. *FEBS Letters, 574*, 176–180.